Impact of eNOS polymorphisms on red blood cell aggregation in sickle cell disease

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Letter to the Editor

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To the Editor,

Hemorheological abnormalities have been well characterized in sickle cell disease (SCD) and seem to modulate the clinical severity [1]. For instance, increased blood viscosity raises the risk for frequent vaso-occlusive crisis (VOC) and increased red blood cell (RBC) aggregates robustness (i.e., RBC disaggregation threshold) enhances the risks for acute chest syndrome (ACS) [2]. Moreover, patients with the most rigid RBCs are characterized by high hemolytic rate, which increases the risks to develop leg ulcers or glomerulopathy [3]. However, except few studies supporting an effect of α-thalassemia and oxidative stress [1] on RBC rheological properties of SCD patients, very few experiments focused on the genetic and cellular factors modulating blood rheology in this disease.

Recently, a growing interest has been devoted to the role of nitric oxide (NO) on blood rheology in healthy individuals: NO would improve RBC deformability and decrease RBC aggregation [4]. Several variants, namely -786-TC polymorphism (rs2070744), 27 pb-VNTR repeat, 894-GT (rs1799983), have been described for the endothelial NO-synthase (eNOS) gene. These variants affect both NO production and endothelial function. One could suggest a relationship between eNOS polymorphisms, plasma NO content and hemorheological characteristics. This hypothesis is supported by the findings of Fatini et al. [5] who found an association between eNOS polymorphisms and RBC rheological properties (i.e. aggregation and deformability) in healthy individuals. However, such phenotype/genotype relationship has never been studied in SCD. The aim of the present study was to highlight eNOS polymorphisms effect on hemorheological parameters and NO levels in SCD.

SCD patients followed at the Sickle Cell Unit of Guadeloupe were included: 110 children (8-16 yrs old) and 131 adults (i.e. ≥ 18 yrs old); 138 with homozygous SCD and 103 with sickle cell hemoglobin C disease. The study was approved by the Regional Ethics Committee (DIRC Sud/Ouest Bordeaux/DOM, registration number: 2009-A00211-56/SAPOTILLE and 2010-A00244-35/HTA projects). All patients provided written informed consent and were at steady state (i.e. no blood transfusion or acute events in the previous three months). Hemorheological parameters were measured as previously described [2]. Plasma NO metabolites (NOx) level was measured in the pediatric cohort [6]. Genomic DNA was

extracted from peripheral blood leukocytes and genotyped using PCR-based methods. Mann–Whitney/Kruskal–Wallis tests were used to compare each parameter between patients classified according to eNOS genotypes. Known parameters affecting hemorheological properties such as age, SCD genotype and hydroxycarbamide treatment were included as covariates in a linear regression model. A $p < 0.05$ value was considered significant.

Baseline characteristics of sickle cell patients for clinical, biological, and hemorheological parameters are presented in Table 1. The genotypic and allelic distributions for three polymorphic variants of the eNOS gene (i.e., VNTR-27pb, 894G>T and -786T>C) are also summarized. The homozygous wild types occur at higher frequency for VNTR-55, -786-TT and 894-GG polymorphisms, respectively (43.7 %, 69.8 %, 84.2 %). The lower frequencies were found for the homozygous mutants: 0.4 % for VNTR-66, 0.4 % for -786-CC, and 0.9 % for 894-GG.

The RBC disaggregation threshold was significantly lower in individuals with VNTR-56 or 66 genotypes compared to 55 or 45 genotypes (Table 2). Linear regression analysis revealed that VNTR polymorphisms significantly affected the RBC disaggregation threshold variability ($p = 0.039$) independently of age ($p = 0.647$), SCD genotype ($p = 0.324$) and hydroxycarbamide treatment ($p = 0.249$). No relationship was detected between eNOS polymorphisms and the other parameters. Although, the 27 pb-VNTR variant harboring 4 copies in intron 4 acts as a cis-acting regulator of eNOS expression and was previously found to be associated with a decreased eNOS expression and NO plasma level [7], this study did not detect any association between eNOS polymorphisms and NOx level in the pediatric cohort. These unexpected results need to be confirmed in larger cohorts of SCD patients but one may underline that chronic hemolysis might have affected plasma NOx level too [8].

We demonstrated for the first time an impact of eNOS 27 bp-VNTR polymorphism on the RBC disaggregation threshold variability in a large cohort of SCD patients. It is worthwhile to notice that faster RBC aggregation was previously described in subjects with the VNTR-44 or 45 genotype compared to those with the VNTR-55 genotype [9]. Altogether, these data suggest that the VNTR polymorphism modulate RBC aggregation properties. These findings may be of clinical relevance since it has been previously demonstrated that increased RBC aggregates robustness enhances the risks for acute chest syndrome (ACS) [2]. Further studies are warranted to test this hypothesis.

Author contributions

Designed the study, supervised research and wrote the manuscript: MR, PC, SF and MDHD

Performed genotyping: LB, SF and MR

Statistical analysis: SF and BT

Performed hemorheological measurements: PC, YL, XW and KCC

Recruited patients and collected clinical data: NL and MEJ

Acknowledgments

We sincerely thank the sickle cell patients who accepted to participate in the study and the medical staff of the "Unité Transversale de la Drépanocytose" at the University Hospital of Pointe-à-Pitre. This study was supported in part by the Region Guadeloupe.

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Table 1. Baseline characteristics of the studied population (n = 241)

Clinical data	**Age** (year)		
		mean ± sd	24.8 ± 15.6
		median *(iqr)*	19 *(24)*
	Sex, n *(%)*		
		Men	111 *(46.1)*
		Women	130 *(53.9)*
	SCD Status, n *(%)*		
		SS	138 *(57.3)*
		SC	103 *(42.7)*
	HU[a] treatment, n *(%)*		
		treated	33 *(13.7)*
		non-treated	208 *(86.3)*
Biological parameters	**NOx**[b] (µmol.l^{-1})		
		mean ± sd	30.8 ± 10.4
Hemorheological parameters	**EI**[c] (a.u.)		
		mean ± sd	0.396 ± 0.1
		median (iqr)	0.408 *(0.131)*
	AI[d] (%)		
		mean ± sd	49.3 ± 9.8
	γ^e (s^{-1})		
		mean ± sd	282.3 ± 112.9
		median (iqr)	275 *(155)*
	Blood viscosity (cP) *mean ± sd*		
		225s	6.3 ± 1.4
		90s	8.2 ± 2.3
eNOS Polymorphisms	**VNTR**, n *(%)*		
		44	21 *(8.8)*
		45	101 *(42.1)*
		55	105 *(43.7)*
		56	12 *(5.0)*
		66	1 *(0.4)*
	-786TC, n *(%)*		
		TT	162 *(69.8)*
		TC	69 *(29.8)*
		CC	1 *(0.4)*
	894GT, n *(%)*		
		GG	198 *(84.2)*
		GT	35 *(14.9)*
		TT	2 *(0.9)*

[a]Hydroxyurea, [b]Nitric oxide end-products, [c]Elongation Index (i.e., RBC deformability), [d]RBC Aggregation Index, [e]RBC Disaggregation threshold

SCD patients: sickle cell anemia patients (138) and sickle cell hemoglobin C patients (103) ;

VNTR: variable number of tandem DNA repeats

Quantitative data are expressed as mean (± standard deviation/±sd) or median (interquartile range/iqr)

Qualitative date are expressed as number *(percentage/%)*

Table 2. Descriptive statistics of biological and hemorheological parameters according to eNOS polymorphisms

	NOx[a] (µmol.l^{-1})			**Blood viscosity 225 s^{-1}** (mPa.s^{-1})			**Blood viscosity 90 s^{-1}** (cP)			**EI**[b] (au)			**AI**[c] (%)			**γ**[d] (s^{-1})		
	n	mean	± sd	n	mean	± sd	n	mean	± sd	n	mean	± sd	n	mean	± sd	n	mean	± sd
VNTR																		
44 + 45	54	30.5	± 9.8	116	6.4	± 1.4	118	8.4	± 2.3	120	0.400	± 0.1	119	50.4	± 9.6	119	291.7	± 121.2
55	50	31.0	± 11.4	96	6.2	± 1.5	95	7.9	± 2.3	101	0.388	± 0.1	101	48.3	± 10.0	101	282.6	± 104.0
56 + 66	4	31.2	± 2.8	13	6.5	± 1.3	13	8.6	± 2.2	13	0.406	± 0.1	13	47.6	± 9.2	13	208.2	± 54.3
*p-value**		*NS*			*NS*			*NS*			*NS*			*NS*			*0.015*[‡]	
-786TC																		
TT	72	31.1	± 10.0	151	6.3	± 1.4	152	8.2	± 2.3	159	0.393	± 0.1	159	49.0	± 9.2	159	282.1	± 110.2
TC + CC	36	32.1	± 11.2	67	6.3	± 1.5	67	8.0	± 2.3	68	0.401	± 0.1	67	49.8	± 11.0	67	286.1	± 121.7
*p-value**		*NS*			*NS*			*NS*			*NS*			*NS*			*NS*	
894GT																		
GG	91	30.7	± 10.2	186	6.4	± 1.4	187	8.2	± 2.3	193	0.396	± 0.1	193	49.3	± 9.7	193	284.8	± 117.8
GT + TT	17	31.2	± 11.9	34	6.1	±1.5	34	8.0	± 2.4	36	0.396	± 0.1	35	48.1	± 11.0	35	271.8	± 90.0
*p-value**		*NS*			*NS*			*NS*			*NS*			*NS*			*NS*	

[a]Nitric oxide end-products measured in the pediatric cohort only, [b]Elongation Index (i.e., RBC deformability), [c]Red blood cell Aggregation Index, [d]Red blood cell disaggregation threshold.

**by univariate analysis*

[‡]p value = 0.039 for RBC disaggregation threshold adjusted for age, SCD status and HC treatment (multivariate analysis)